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AQUEOUS FLUID DYNAMICS IN AVIAN GLAUCOMA

BY

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A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Aqueous fluid dynamics in avian glaucoma", submitted by Elliot N. Frankelson, M.D., in partial fulfilment of the requirements for the degree of Master of Science (Surgery).

ABSTRACT

Chickens reared under conditions of continuous light from the time of hatching develop a glaucoma-like condition characterized by increased eye weight and size, elevated intraocular pressure, decreased aqueous outflow facility, flattening of the cornea, and a shallow anterior chamber with a narrow iridocorneal angle.

In this study, a series of experiments were carried out in an attempt to explore the etiology of this light-induced avian glaucoma.

In Part I Diamox (acetazolamide) was given orally, to prevent glaucoma in birds simultaneously exposed to continuous light. This drug, which is known to decrease aqueous secretion and thus decrease intraocular pressure (IOP), reduced the severity of the glaucoma which developed. This suggests that increased IOP, which may be secondary to hypersecretion of aqueous humor and/or to a defect in outflow facility, may be a primary factor in the development of light-induced avian glaucoma.

In Part II of this study, the role of angle closure in the development of light-induced avian glaucoma was examined. Iridectomies, performed on young chicks which were subsequently exposed to continuous light, did not prevent avian glaucoma. This suggests that angle closure is not the primary mechanism responsible for light-induced avian glaucoma.

Part III reports studies on the effect of acute intravenous Diamox on aqueous humor dynamics in normal and glaucomatous chickens. In normal birds, the Diamox caused a marked decrease in aqueous secretion and outflow facility, but only a transient drop in intraocular pressure. In glaucomatous birds, Diamox caused a marked decrease in aqueous secretion, an increase in aqueous outflow facility, and a marked and sustained drop in intraocular pressure. These results suggest that light-induced avian glaucoma is associated with some disturbance in the homeostatic mechanism which regulates intraocular pressure.

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I. INTRODUCTION

In humans, glaucoma is an eye disease which is characterized by an increased intraocular pressure, which produces arcuate defects in the field of vision resulting from retinal nerve fiber bundle damage often associated with excavation of the optic disc.

It is now well established in the literature that a condition similar to human glaucoma develops in chickens exposed to continuous light from the time of hatching. Enlargement of the eyes of chickens exposed to continuous incandescent light was first reported by Jensen and Matson in 1957.[55] This observation was confirmed and more extensively studied by Lauber and co-workers.[69]

This light-induced avian glaucoma is characterized by buphthalmos, increased intraocular pressure, decreased aqueous outflow facility, shallow anterior chamber, narrow iridocorneal angle, and flattening of the cornea. After prolonged light exposure, pathological changes develop in the retina and ciliary body, there is fibrous metaplasia of intraocular structures, and intraocular bone formation may occur.[68]

The purpose of this study was to investigate the etiology of light-induced avian glaucoma by noting the effect of Diamox or surgical iridectomy on the development of this condition.

II. NORMAL AQUEOUS HUMOR DYNAMICS

A. The Aqueous Humor

The following are based on the currently accepted picture for the eyes of humans and certain laboratory animals, and are presumed to be similar for the chicken.

The anterior and posterior chambers of the eye are filled with aqueous humor, a crystal clear liquid having a refractive index which is relatively low compared to that of the lens which it bathes.

The lens of the eye, and to some extent, the cornea, are devoid of blood vessels, and nourishment is supplied to these structures by the aqueous humor.

The aqueous humor also transports oxygen into the interior of the eye and carries away the waste products of metabolism into the blood.

1. Chemical Composition of Aqueous Humor

a. Protein. The aqueous humor is a more dilute fluid than the blood serum. In man it contains 1.08 gm/100 cc solid as compared to the blood serum which contains 9.5 gm/100 cc solids. The chief difference in solids is due to the lower percentage of protein present in the aqueous.[40,57]

b. Amino acids. Analyses by Merriain[76] using starch column chromatography, indicate that the distribution of amino acids in the aqueous humor is similar to that of the free amino acids in the blood plasma.

c. Organic acids.

(1) Ascorbic acid. The concentration of ascorbic acid (Vitamin C) in aqueous humor is significantly higher than in the circulating plasma in some animals, including man.[78] This higher value may be partly due to diffusion of ascorbic acid from the adjacent tissues, but present evidence favours active transport across the blood-aqueous barrier as the major route.[59,83]

(2) Uric acid. Uric acid is present in the aqueous humor in a slightly lower concentration than in blood serum.

(3) Lactic acid. The lactic acid content of the aqueous humor may be slightly higher than that of plasma. This higher content is probably due to the metabolic activity of all the tissues bordering the anterior and posterior chambers.[58]

(4) Hyaluronic acid. This substance is present in the aqueous humor, the vitreous, and in the drainage channels of the iridocorneal angle, but not in the blood stream. It may be elaborated in the vitreous humor or secreted by the ciliary body.[77]

(5) Carbonic acid. In the human the pH and the concentration of bicarbonate are lower in the aqueous humor than in the plasma,[9] but there is a considerable variation between different species.[30]

d. Inorganic ions.

(1) Sodium. Most investigators agree that the concentration of sodium ions in the anterior chamber is slightly less than in the plasma.[31,33,61]

Substance	Anterior Aqueous Humor (mM/kg H_2O)		Posterior Aqueous Humor (mM/kg H_2O)		Plasma (mM/kg H_2O)	
Anions						
Chloride	105.1	(73)†	100.0	(72)	111.8	(42)
Bicarbonate	27.7	(23)	34.1	(23)	24.0	(23)
Lactate	12.1	(24)	11.2	(24)	8.2	(12)
Ascorbate	0.96	(16)	1.30	(16)	0.02	(5)
Phosphate	0.89	(26)	0.52	(22)	1.49	(13)
Cations						
Sodium	146.5	(34)	144.5	(34)	--	(2)
Potassium	1154§	(4)	1307.0	(4)	1545.0	(2)
Nonelectrolytes						
Carbon dioxide	1.0	(23)	1.2	(23)	1.2	(23)
Nonprotein nitrogen	13.4	(25)	13.5	(25)	17.6	(16)
Urea	6.3	(46)	5.8	(46)	7.3	(23)
Glucose	6.7	(22)	7.1	(22)	8.3	(11)

continued

† Figures in parentheses refer to number of cases
§ Expressed as counts per minute per kilogram
* From Kinsey, V.E.: Arch. Ophthal. (Chicago), 50:401, 1953.

Table I. Summary of composition of plasma and aqueous humor of anterior and posterior chambers of rabbit eye.*

Substance	Anterior aqueous humor/posterior aqueous humor	Anterior aqueous humor/plasma	Posterior aqueous humor/plasma	Anterior aqueous humor in excess of posterior aqueous humor (mM/kg H_2O)
Anions				
Chloride	1.051	0.94	0.89	+5.1
Bicarbonate	0.81	1.15	1.42	-6.4
Lactate	1.09	1.47	1.37	+0.9
Ascorbate	0.75	48.0	65.0	-0.3
Phosphate				
Cations	1.80	0.60	0.36	+0.4
Sodium	1.01	-	-	+2.0
Potassium	0.89	0.75	0.85	-0.5 (estimate)
Nonelectrolytes				
Carbon dioxide	0.83	0.83	1.0	-0.2
Nonprotein nitrogen	0.99	0.76	0.77	-0.1
Urea	1.08	0.86	0.80	--ξ
Glucose	0.96	0.81	0.85	-0.4

ξ Figures for urea are omitted since they are included in values for nonprogein nitrogen

Table I. Continued.

(2) Chloride. Chloride values vary greatly in different species. In the rabbit there is a deficit of chloride in the aqueous as compared with plasma while the reverse is true in man, who has a surplus of chloride in the aqueous humor.

(3) Calcium. The calcium level in the aqueous humor is lower than that in the vitreous humor which is in turn still lower than that in the blood serum.[86]

2. The Formation of Aqueous Humor

The dynamics of the aqueous humor is concerned with the movement of various components of plasma, together with substances which may be manufactured by the tissues of the eye, into and out of the posterior and anterior chambers, and with the forces associated with this movement.

Experimental evidence indicates that normally aqueous fluid is constantly flowing through the eye. The fluid arises in large part from the ciliary body behind the iris, fills the posterior chamber, flows through the pupil into the anterior chamber, and leaves the eye via the drainage apparatus at the iridocorneal angle.

In addition to this bulk movement of fluid, there is a thermal convection circulation of aqueous which is the result of differences in temperature between the various regions of the anterior chamber.

There is thus a constant circulation of aqueous humor within the anterior chamber as well as the bulk flow of aqueous humor into and out of the eye.

Theories of Formation of Aqueous Humor

a. Ultrafiltration. Leber[1] first proposed, in the early twentieth century, that the capillary bed in the ciliary body produces aqueous humor by ultrafiltration. He believed that the osmotic pressure of the aqueous humor was lower than that of the blood by 30 mm Hg, mainly due to the absence of protein in the aqueous. As the capillary wall prevents large protein molecules from going into the aqueous, there should be a mass movement out of the blood of solutes such as sodium, chloride, urea, and glucose, which are small enough to pass through the capillary endothelium, along with water as a solvent.

If this were the case, freely diffusible substances such as urea and sugar should be found in equal concentrations in both aqueous humor and blood, but chemical analysis does not bear this out. Not only are certain substances such as glucose and urea deficient in the aqueous humor, but it has been shown that aqueous as compared to blood, shows a higher concentration of bicarbonate in rabbits, of chloride in man, and of ascorbic and hyaluronic acids in both species.

Some other mechanism in addition to ultrafiltration must account for the formation of aqueous humor, although filtration is considered to be part of the process.

b. Dialysis.[1] This theory assumes that the aqueous humor is in equilibrium with blood, and postulates that the

hydrostatic pressure in the capillaries merely balances the intraocular pressure. The aqueous humor becomes, in this view, a static fluid, and the interchange between the blood and the aqueous is therefore molecular.

In order to meet the theoretical requirements for dialysis, a fluid so formed must have the non-electrolytes in equal concentration in the parent fluid and in the dialysate. The dissociated electrolytes, whose distribution is dependent on their net charge, must arrange themselves on the two sides of the membrane according to the requirements of the Gibbs-Donnan Equilibrium.

The chief evidences against the formation of aqeuous humor by dialysis are the excess of certain substances such as ascorbic and hyaluronic acid in the aqueous, and the deficiency of others such as urea and glucose. There is also evidence that aqueous humor is not a static fluid, but that there is a constant through and through circulation of considerable magnitude.

c. Secretion.[1] Secretion is the term used to describe the process by means of which water and other substances are transferred across a membrane against a gradient, at the expense of cellular energy. This is also called active transport. That secretion may be involved in the production of aqueous humor is suggested by excess of certain substances such as ascorbic acid in the aqueous as compared to blood.

There are two main theories which attempt to explain how this active transport may occur:

1. Redox-Pump theory. Friedenwald[42,43] postulated a barrier between the epithelium and stroma of the ciliary body, with an electron transport system working across this barrier. Oxidation of a substrate on the stromal side of the barrier is coupled with reduction of an electron carrier on the epithelial side. This is the first of a series of oxidation-reduction reactions across the barrier to the epithelial side where the cytochrome system has the capacity of using these electrons to add hydrogen to oxygen. The resulting hydroxyl ions react with the carbonic acid to produce bicarbonate ions in the epithelium and aqueous humor. The H^+ ions left on the stromal side would also be buffered by the bicarbonate system.

Both of these buffering systems would be more efficient in the presence of carbonic anhydrase. In its absence or effective inhibition, the secretory site or step most sensitive to alterations in pH would be impaired.[10] This theory has led to the use of carbonic anhydrase inhibitors such as Diamox to suppress aqueous secretion.

2. Sodium-potassium activated ATPase and active cation transport.[24] The ciliary epithelium and the lens epithelium have both been shown to contain sodium-potassium activated ATPase. In order to function properly, both the enzyme and the cation transport system require sodium and potassium. The system is inhibited by cardiac glycosides

such as digitalis, the degree of suppression of aqueous humor secretion paralleling the amount of enzyme inhibition.[92,14]

According to this theory, water enters passively with the salt, and the enzyme carbonic anhydrase probably plays an indirect role, such as maintaining cellular pH in the ciliary epithelial cells.

d. Secretion-Diffusion Theory. It has been shown that the total production of aqueous humor cannot be accounted for by filtration and/or dialysis. The flow process and the chemical and osmotic differential between aqueous and blood suggest that energy must be expended.

The theory that is most widely accepted at the present time is that the aqueous humor is formed by a combined process of diffusion and secretion, and that much of the water enters by secretion and carries the solutes along with it.

3. The Normal Rate of Flow of Aqueous Humor.

In the normal eye there are variations in the rate of aqueous flow related to such factors as aging, endocrine disturbances, state of hydration, influence of certain drugs, and surgery. These fluctuations are usually of small magnitude and are accompanied by what appear to be compensatory changes in outflow facility so that a relatively constant intraocular pressure is maintained.[62]

The rate of aqueous flow in human eyes varies spontaneously in a diurnal manner, and this largely accounts for the diurnal fluctuations in intraocular pressure.[11,38] The variations have been correlated with diurnal changes in plasma corticosteroid levels, both being higher by day than by night.[25]

The rate of aqueous flow may be expressed mathematically if one knows the values for the head of pressure which maintains the flow and for the resistance which the fluid meets on its way through the outflow channels. This formula was published by Goldmann in 1947.[47]

$F = \frac{\Delta P}{R}$ where:

F = outflow (μL/min)

R = resistance to outflow

ΔP = the head of pressure in mm Hg

If one is able to determine the volume of flow (F) per minute, the intraocular pressure (P), and the pressure in the aqueous veins (Pv), the equation $R = \frac{(Po - Pv)}{F}$ expresses the resistance in the outflow system between the anterior chamber and the episcleral venous plexuses. This is taken to be the actual resistance to the outflow of fluid from the eye.

If R is considered to be the inverse of C which is Grant's Coefficient of outflow facility, the formula is expressed $C = \frac{F}{Po - Pv}$ and flow can be determined by the formula $F = C(Po - Pv)$.

4. Normal Outflow Facility and its Variations

The mean value for C as measured tonographically in a large population of normal humans is 0.28 ± 0.05.[51]

a. Site of Resistance. Grant demonstrated, by careful dissection, in enucleated normal eyes that some 75% of the resistance to outflow is in the trabecular meshwork.[12]

In vitro, in rabbit eyes, it has been demonstrated that a part of the resistance to outflow can be decreased by hyaluronidase injected into the anterior chamber.[8] The large amount of acid mucopolysaccharides present could provi de a mechanism for the control of outflow resistance. They may be formed locally by endothelial cells of the trabecular meshwork or may be filtered from the aqueous humor, being carried from the vitreous humor or from the internal limiting membrane on the surface of the ciliary epithelium.[22,96,99]

b. The influence of sympathetic innervation.

Twenty-four hours after excision of the superior cervical ganglion (sympathetic) in rabbits, there is an increase in C and a decrease in IOP, presumably due to the release of alpha-adrenergic substances into the anterior chamber.[64] After one week C drops below normal and no catecholamines can be detected in the ciliary body. The outflow mechanism of such eyes is very sensitive to l-noradrenaline, and reacts to small amounts of this agent injected into the anterior chamber by large increases in C.[89,90,91]

c. Influence of endocrine factors. The variations of C with the menstrual cycle and the large increase in C during pregnancy suggest direct or indirect endocrine involvement in control of outflow.

This is also suggested by the decreased C and increased IOP seen in some patients when topical steroids are applied to the eye.[3,4,16]

d. Glaucoma. It is well documented that C is decreased in glaucoma.[34,50,51]

In angle closure glaucoma, this impairment results from obstruction to outflow by the approximation of the iris and the cornea, thus blocking the trabecular meshwork.[54,97]

In glaucomatous eyes with open angles, the obstruction is probably in the trabecular meshwork itself.

Where the trabecular meshwork borders Schlemm's canal, various degrees of hypercellularity and vacuolated appearance have been demonstrated. In more advanced cases, the trabecular sheets become thickened and hyalinized and the spaces between them are narrowed.[13,54,95]

5. The Intraocular Pressure (IOP)

The normal mean IOP in man is 15.4±2.5 mm Hg.[46,97] During a 24 hour period, the IOP normally varies in a characteristic fashion. The IOP is lowest at about 5 to 7 o'clock in the evening and gradually rises during the night, reaching a peak in the early morning and then gradually decreasing during the day. In normal eyes, the diurnal

variation in IOP seldom exceeds 3 or 4 mm Hg, but in chronic open angle glaucoma, there may be much greater variation.[32]

There is some disagreement regarding the factors which determine normal diurnal changes in IOP. Duke-Elder feels that the diurnal variations are caused by variations in outflow facility (C), due in turn to pressure changes in the efferent veins brought about by changes in sympathetic tonus.[35]

Other evidence suggests that diurnal changes are associated with changes in secretory activity of the ciliary body.[38,49,71]

Factors Associated with the Maintenance of Intraocular Pressure.

a. Rigidity of the Sclera. In humans and experimental animals, the sclera is distensible at low pressures, but as the IOP increases the distensibility of the sclera decreases.[28,36,37,80,84]

b. Intraocular contents. The following contents of the eye contribute to the maintenance of normal IOP:

(1) Solid structures. All of the solid and semi-solid intraocular structures such as the lens, vitreous, iris, uveal tract, and retina, aid in maintaining normal IOP and any of these may, by a change in volume, cause an alteration in the IOP.[94]

(2) Fluid contents. There may be alterations in the

IOP due to variation in the quantity and quality of the blood.

The volume of blood in the intraocular blood vessels accounts for a part of the volume of the intraocular contents. The state of contraction or dilation of these blood vessels will determine the volume of blood in the interior of the eye at any one time, and will thus alter the IOP. It follows that any factor which affects the filling of these vessels, such as arterial blood pressure,[7] venous pressure in the head,[72] or blood flow through the eye,[23] may produce changes in IOP.

(3) Alterations in IOP due to formation and elimination of aqueous humor. As a result of the constant accumulation of aqueous humor in the posterior chamber and the constant exit of this fluid from the anterior chamber,the volume of the eye may change. When these opposing processes are in balance, and IOP does not change, the quantity of fluid coming into the eye from the blood is presumably equal to the quantity leaving the eye per unit time.[5]

The IOP will be increased if either the rate of formation of fluid is increased or the rate of outflow is decreased. Conversely, the IOP will decrease if the rate of formation is decreased or the rate of outflow is increased.[63]

(4) Hypothermia. Lowering the body temperature in experimental animals will produce a marked decrease in the rate of formation of aqueous humor and a resulting decrease in IOP.[15]

Outflow facility is also decreased by hypothermia, probably due to the increased viscosity of the aqueous humor at low temperatures. However, the decrease in aqueous production is greater than the decreased outlfow so that the net result is a decrease in IOP.[81]

III. THE PHARMACOLOGY OF ACETAZOLAMIDE (DIAMOX)[48]

One phase of the present study involved use of the carbonic anhydrase inhibitor, acetazolamide. The following is a discussion of the pharmacology of this drug.

The enzyme carbonic anhydrase catalyses the formation of bicarbonate from the carbon dioxide given off during cellular respiration:

$$CO_2 + H_2O \overset{I}{\rightleftharpoons} H_2CO_3 \overset{II}{\rightleftharpoons} H^+ + HCO_3^-$$

The hydration or dehydration reaction (I) is catalyzed by the enzyme carbonic anhydrase. Reaction II is an ionic dissociation that is virtually instantaneous and not subject to enzymatic acceleration.

Carbonic anhydrase has been found in many sites such as red blood cells, renal cortex, gastric mucosa, pancreas, central nervous system, and at several locations in the eye. The enzyme is widely distributed in these different tissues and is not localized to a single cellular fraction.

1. Pharmacologic actions. Mechanism of action. The major pharmacological action of acetazolamide is the non-competitive inhibition of the enzyme carbonic anhydrase. In general,

carbonic anhydrase is present in the tissues in great excess. More than 99% of enzyme activity in the kidney must be inhibited before physiological effects become apparent.

2. Effect on the eye.

It was postulated by Friedenwald[44] and by Kinsey[58] that carbonic anhydrase is necessary for the secretion of bicarbonate ion from the blood into the aqueous humor. It was subsequently shown that acetazolamide will cause a partial suppression of the secretion of aqueous humor and a resultant decrease in IOP.[17]

Rabbits and guinea pigs have a high concentration of bicarbonate in their aqueous humor. In these animals, Diamox causes a rapid fall in the amount of bicarbonate in the aqueous humor which is well correlated with a decrease in secretion of aqueous humor and lowering of the intraocular pressure.[2]

It is more difficult to attribute the pressure-lowering effect of acetazolamide to inhibition of carbonic anhydrase in those species, including man, in which bicarbonate is not present in excess in the aqueous humor when compared to blood plasma.

Becker showed that in man acetazolamide also reduces the excess of chloride in the aqueous, increases bicarbonate, and thus raises pH.[18,19] He suggested that acetazolamide may act by reducing the buffering capacity of the individual secretory cells, thus leading to a partial suppression of secretion.[20]

Macri[73] found that the IOP at a steady state is dependent upon the venous pressure, and that a direct relationship exists between venous pressure and iris artery pressure.[74] His studies showed that acetazolamide produces a sustained increase in resistance to perfusate flow through the iris arteries in the cat. From these results, he concluded that the action of acetazolamide in decreasing IOP is due to a decrease in the bulk volume within the eye which is caused by a constriction of the arterioles supplying the iris and ciliary body.

Kass and Green[53,56] found the osmotic pressure of the posterior chamber aqueous to be nearly equal to that of plasma, whereas the anterior chamber aqueous was considerably hypertonic. Acetazolamide decreased the osmotic pressure of anterior chamber aqueous, but did not affect posterior chamber fluid.

From these results they conclude that the effect of acetazolamide does not involve changes in the osmotic pressure relationships of aqueous humor as it is formed from the blood in the ciliary processes.

Thus, it can be seen that the exact mechanism of action of acetazolamide is unclear, although it seems certain that the drug decreases aqueous secretion.

IV. METHOD USED TO MEASURE INTRAOCULAR PRESSURE, AQUEOUS OUTFLOW FACILITY, AND AQUEOUS INFLOW.

The techniques used in this study are as previously described by Lauber, Boyd and Boyd.[65]

1. Method Used to Measure Intraocular Pressure (IOP)

The chicks were anesthetized with intramuscular Nembutal (50 mgm/Kg) and placed in a holding apparatus designed to immobilize the head (Fig. 1). Both eyes were then cannulated from the posterior side with #27 needles connected via polyethylene tubing to Statham P23-Dd pressure transducers which were in turn connected to Beckman type R amplifier-recording system in order to obtain a continuous recording of IOP.

One eye was allowed to equilibrate for at least one hour after cannulation. By this time, the IOP was usually stable and this was considered to be the pre-infusion intraocular pressure (Po).

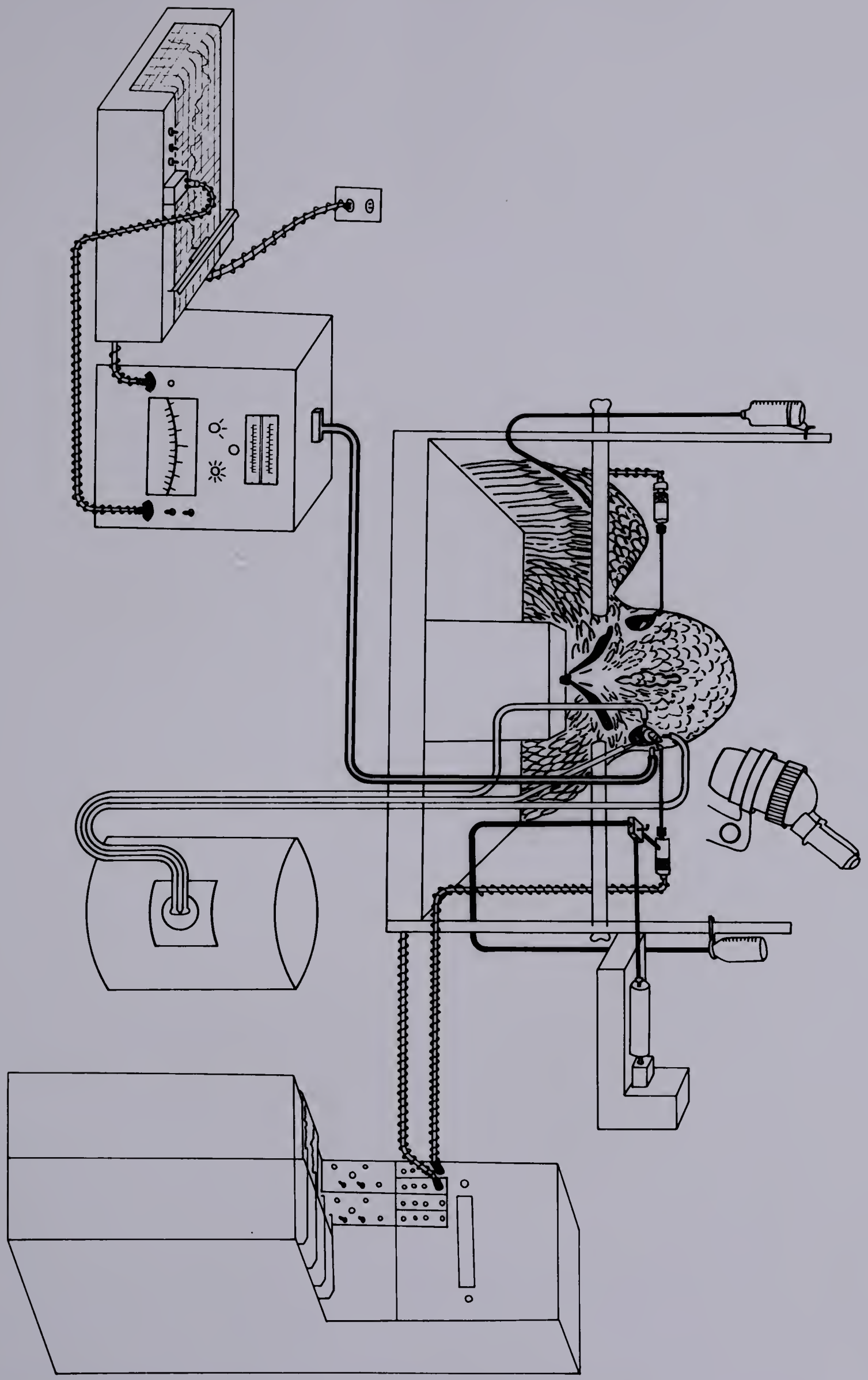
2. Method to Determine Aqueous Outflow Facility (C).

After the IOP had stabilized, normal saline was infused at a constant low rate. The saline infusion produced an increase in IOP which reached a plateau (Pi). At this point the saline infusion was stopped, the IOP was allowed to return to pre-infusion level for at least 15 minutes, and a repeat infusion was carried out at the same rate. The value of C was determined by taking the average of the results of three infusions.

Figure 1*

Experimental arrangement for monitoring aqueous flow. A cannual connects each anterior chamber to a pressure transducer, whose signals are recorded on a Beckman Dynograph recorder (lower right). A Harvard syringe pump (upper right) is connected to the system on one side to provide for saline infusion during intraocular pressure recording. Near the Harvard pump is a saline reservoir for calibration of the transducers. An indwelling wing vein cannula (upper left) is connected to a drip infusion apparatus for administration of fluorescein. The bird's right eye serves as a control. Near the experimental eye are three optic fibers carrying ultraviolet light from a mercury arc lamp (below, center) at the activation wavelength for fluorescein, 365 mμ. Also approximating the left eye is another optic fiber (shown with dark borders) which conducts light from the eye to a spectroradiometer (lower left), where it is measured and recorded at the fluorescence wavelength for fluorescein, 530 mμ. A zoom stereomicroscope is available for observation and photographic recording of the events in the experimental eye.

*Lauber, J. K., Boyd, T. A. S. and Boyd, J. Canad. J. Ophthal., 4:55, 1969.



Becker's Formula[90] was used to calculate C:

$$C = \frac{I}{Pi - Po}$$ where: C = outflow facility (μL/min/mmHg)

I = infusion rate (μL/min)

Po = pre-infusion IOP (mmHg)

Pi = IOP at plateau during infusion (mmHg)

3. Method to Measure Aqueous Inflow

The method used to measure aqueous flow was the fluorescein technique as described by Lauber *et al.*[65]

a. Materials used. Sodium fluorescein is a small molecule which is felt to be physiologically inactive and able to enter and leave the eye easily. It fluoresces strongly with a brilliant green colour when illuminated by light in the near ultraviolet part of the spectrum.

Ultraviolet light of 365 millimicrons wavelength was used to illumiante the eye and was conducted from the light source to the eye by means of an optic fiber system. The fluorescence from the eye was detected by an optic fiber probe which was aimed at the pupil and placed within 1 cm from the cornea. This probe was connected to a spectro-radiometer* with the wavelength selector set at 530 milli-microns, the wavelength of fluorescence of fluorescein. The spectroradiometer measures light in units of energy rate intensity per spectral bandwidth.

The signal from the spectroradiometer is amplified and recorded on a 10 millivolt strip chart recorder.

*ISCO, Model SR

b. Method. One cc of 10% sodium fluorescein was injected into a plastic catheter which had been previously placed in a wing vein. This catheter was connected to a reservoir bottle of 10% fluorescein via a drip chamber and the fluorescein level in the blood was kept at a fairly constant level by supplementing it from the reservoir of fluorescein at the rate of 6 to 10 cc/hour.

Fluorescein is usually detected in the anterior chamber within 30 seconds after intravenous injection, and continues to build up until it reaches a plateau in 10 to 20 minutes.

By using the record of fluorescein build-up in the eye, the percentage of "non-fluorescein" is plotted against time to obtain an exponential decay curve which becomes a straight line when plotted on semi-logarithmic paper.

If one knows the volume of the aqueous space and the time taken to accomplish one complete exchange of aqueous, flow can be calculated from the formula:

$F = \frac{V}{T}$ where: F = aqueous flow (μL/min)

V = volume of aqueous space (mm^3)

T = 1/2 life of non-fluorescein in the aqueous space (min)

The calculation of volume of the aqueous space assumes that the cornea is a segment of a perfect sphere:

$V = \frac{1}{6} \pi h(h^2+3a^2)$ where:

V = volume of the aqueous space (μlitres)

h = depth of the anterior chamber (mm)

a = radius of the cornea (mm)

This method gives a value for aqueous flow which may be expected to be slightly lower than the actual rate of inflow because some of the fluorescein escapes from the eye via the usual outflow channels during the period of build-up of fluorescein in the aqueous.

It can be seen that this mehtod of measuring inflow does not require prior determination of C, and can be used to measure aqueous inflow in the undisturbed eye.

V. EXPERIMENTAL STUDIES IN LIGHT-INDUCED AVIAN GLAUCOMA

Part I: The Role of Hypersecretion of Aqueous Humor in Light-Induced Avian Glaucoma.

Introduction

Lauber, Boyd, and Boyd[66] found that the outflow facility (C) in chicks reared under continuous light (24L/0D) was above normal during the first few weeks after hatching. By 6-8 weeks of age, outflow facility decreased to below normal levels. This decrease in C was later followed by an increase in intraocular pressure. These findings regarding the sequence of events leading to severe glaucoma led Lauber *et al.*[66] to suggest that increased inflow of aqueous could be responsible for the development of light-induced avian glaucoma. If intraocular pressure is maintained by a homeostatic mechanism, variations in aqueous inflow and/or outflow are the most likely controlling factors. The increase in C in preglaucomatous avian eyes may be viewed as

an "attempt" to maintain a normal IOP, in spite of opposing forces initiated by continuous light. At some point around six weeks of age, abnormalities of aqueous humor dynamics begin to appear: C falls to subnormal levels, although IOP does not rise. This suggests that some level of homeostatic control remains, i.e. inflow must be decreased. The present study bears out this prediction, as does more recent work by Lauber *et al.*[66]

Lauber *et al.*[67] kept a group of chicks in continuous light and fed them Diamox for six weeks. Eye weights were lower in such Diamox fed birds in both 24L/0D and 14L/10D lighting treatments. Since Diamox had prevented the expected eye enlargement under continuous light, and since the drug is thought to exert its major effect on aqueous secretion, Lauber concluded that aqueous inflow is the system primarily involved in light-induced avian glaucoma.

Smith, Becker and Podos[93] fed Diamox to chicks exposed to 24L/0D or 12L/12D lighting treatment. They found that the Diamox prevented much of the increase in IOP associated with avian glaucoma, but did not prevent the angle closure, which these authors concluded was responsible for the light-induced lesions.

Materials and Methods

In this part of the study, Diamox was fed to chicks reared under either continuous (24L/0D) or diurnal (14L/10D) lighting treatment. The purpose was to determine what

effect the drug had on the development of light-induced avian glaucoma by measuring several parameters with more sensitive and critical methods than were previously available. Two other groups not fed Diamox were used as controls. A summary of experimental subjects is shown in Table II.

All the birds were kept in identical environmental conditions except for the photoperiod which was either 24L/0D or 14L/10D.

The timing of the photoperiod was controlled with a Paragon time clock and the light source was 2-100 watt incandescent light bulbs.

Feed containing 45 mgm Diamox/100 grams of feed was given to Group I and Group III chicks from the time of hatching, while Group II and Group IV birds had no Diamox in their feed. Food (chick starter crumbles) and water were supplied *ad lib*.

At 6 and 14 weeks of age, randomly selected birds from each treatment were anesthetized with intramuscular Nembutal (50 mgm/kgm) and IOP, C, and flow were evaluated as previously described.[65]

Results (Tables III, IV, V, VI)

Not all of the characteristic changes of light-induced avian glaucoma were present in the 24L/0D chicks at 6 weeks of age, but by 14 weeks of age marked glaucoma was obvious in all 24L/0D birds (Tables V and VI) (Figs. 2, 3, 4, 5, 6, 7).

(1) Intraocular pressure. There was no significant difference in IOP between any of the groups at 6 weeks of age (Fig. 2). This is in marked contrast to the results obtained at 14 weeks, however, when the IOP in both 24L/0D groups was significantly greater than the IOP in the 14L/10D groups (Fig. 2). ($.001<p<.01$).

The IOP in chicks fed Diamox was not significantly different at 6 weeks within each lighting treatment. By 14 weeks this trend was more pronounced, with IOP being significantly lower in the Diamox-treated birds ($.02<p<.05$).

(2) Aqueous outflow facility (C). Although there was no difference in the C between any of the groups at 6 weeks of age, by 14 weeks there was a marked decrease in C in the 24L/0D chicks. The mean C in the 24L/0D controls was 0.30 ± 0.08 as compared to 2.28 ± 0.11 for 14L/10D controls at 14 weeks (Fig. 4).

The C in 24L/0D birds fed Diamox was greater than that of the 24L/0D birds not fed Diamox, but this difference was not statistically significant (Fig. 5). This tendency for the higher C in the Diamox fed group may be more meaningful when it is correlated with differences in several other parameters (Figs. 3, 6, 7) showing that Diamox had a pronounced effect on aqueous fluid dynamics and on the development of the glaucoma.

(3) Eye weight. There was no difference in eye weight between any of the groups at 6 weeks of age, but by 14 weeks the eye weights of 24L/0D chicks were significantly higher

than the eye weights of the 14L/10D birds ($.001<p<.01$). (Fig. 6). The eyes of 24L/0D Diamox-fed chicks weighed significantly less than those of 24L/0D birds fed a normal diet ($.001<p<.01$). (Fig. 6).

(4) Aqueous flow. The absolute aqueous flow values and statistical comparisons for flow between groups were similar at 6 and 14 weeks of age.

Aqueous flows for the 24L/0D chicks were significantly lower than the flows of the 14L/10D chicks ($.001<p<.01$). (Fig. 7). The flow values for Diamox-fed chicks within each lighting treatment were also significantly lower than that for birds fed a normal diet ($.01<p<.02$). (Fig. 7).

(5) Volume of the aqueous space. The volume of the aqueous space was significantly smaller in the 24L/0D chicks as compared to the 14L/10D chicks, both at 6 and 14 weeks ($.001<p<.01$). It was also interesting to note that the aqueous space was significantly larger in the 24L/0D chicks fed Diamox as compared to the control 24L/0D chicks ($.02<p<.05$). There was, however, no difference in the aqueous space volume between the 14L/0D chicks fed Diamox and the 14L/10D chicks fed a normal diet.

(6) Corneal height. The cornea height is a measure of the height of the space occupied by aqueous humor.

By 6 weeks of age the cornea height was significantly less in the 24L/0D chicks as compared to the 14L/10D chicks. and this effect was still apparent at 14 weeks of age ($.001<p<.01$). It was interesting to note that the cornea

height in the 24L/0D chicks fed Diamox was greater than that in the control 24L/0D birds fed a normal diet ($.01<p<.02$).

(7) Radius of curvature of the cornea. The corneal radius of curvature is an expression of the amount of flattening of the cornea that occurs with eye enlargement. The greater the radius of curvature, the flatter the cornea.

There was a small, although nonsignificant difference in the corneal radius of curvature between groups at 6 weeks, but by 14 weeks the previous trend was marked: 24L/0D chicks had significantly less convex corneas than the 14L/10D chicks ($.01<p<.02$). Diamox did not prevent flattening of the cornea: there was no significant difference between 24L/0D birds fed Diamox and 24L/0D birds fed a normal diet.

(8) Diameter of the cornea. Corneal diameter in 24L/0D chicks was significantly smaller than that in 14L/10D birds both at 6 and 14 weeks ($.001<p<.01$). The mean corneal diameter of the 24L/0D chicks fed Diamox was significantly smaller than that of the 24L/0D chicks fed a normal diet ($.001<p<.01$).

(9) Equational diameter of the eye. The equatorial diameter is the greatest measurement of the eye in the horizontal meridian. At six weeks of age the 24L/0D eyes were not significantly larger than controls in this dimension. By 14 weeks of age the eyes of the 24L/0D chicks had a significantly greater equatorial diameter than those of the 14L/10D chicks ($.01<p<.02$). The eyes of 14-week-old 24L/0D chicks fed Diamox had significantly smaller equatorial diameters

than those of 24L/0D chicks fed a normal diet.

(10) Meridonial diameter of the eye. The meridonial diameter is a measure of length of the eye when viewed from the side. The mean meridonial eye diameter of 24L/0D chicks was significantly greater than that of the 14L/10D chicks by 6 weeks, and this difference was still present at 14 weeks of age ($.02 < p \leq .05$). Oral Diamox did not prevent enlargement of the meridonial diameter of the eyes of 24L/0D birds.

(11) Body weight. There was no significant difference in body weight between the lighting treatment groups at 6 or 14 weeks of age. There was, however, a significant difference in body weight within lighting treatments. At both 6 and 14 weeks of age, chicks fed Diamox were lighter than those fed a normal diet ($.01 < p < .02$). This effect of Diamox on body weight is in agreement with the known effects of the drug in other species.

Conclusions

(1) Glaucoma developed in chickens exposed to continuous light from the time of hatching.

(2) Oral Diamox administered during the period of continuous light treatment altered the course of the developing glaucoma. A less severe form of the disease developed in Diamox-treated birds as compared with controls exposed to continuous light but fed a normal diet.

(3) In view of the known action of Diamox in reducing aqueous secretion and that aqueous hypersecretion has been found to be present in the first few weeks of life during

continuous light treatment,[66] the above results suggest that increased aqueous inflow may be a primary mechanism in the development of light-induced avian glaucoma.

The results of this part of the study are considered more fully in the General Discussion.

	Lighting* Treatment	Diamox in Feed**	Experimental Eye	Control Eye
Group I	24L/0D	Yes	Cannulation Infusion	Cannulation
Group II	24L/0D	No	Cannulation Infusion	Cannulation
Group III	14L/10D	Yes	Cannulation Infusion	Cannulation
Group IV	14L/10D	No	Cannulation Infusion	Cannulation

* 24L/0D = continuous light
14L/10D = 14 hours light, 10 hours darkness

** 45 mgm Diamox per 100 grams of feed

Table II. A summary of experimental subjects for Part I.

	Group	n	Body Weight (kg)	Eye Weight (gms)	IOP Experimental Eye (mmHg)	IOP Control Eye (mmHg)	Outflow Facility (µL/min)	Aqueous Inflow (µL/min)
I	24L/0D Oral Diamox	5	0.741±0.03	1.9±0.01	13.2±1.0	11.8±0.92	2.37±0.31	2.87±0.31
II	24L/0D No Diamox	5	0.976±0.06	2.1±0.1	12.6±0.9	12.9±0.88	1.68±0.23	4.13±0.48
III	14L/10D Oral Diamox	5	0.653±0.02	1.9±0.01	11.5±0.5	10.2±0.44	1.78±0.14	8.43±1.86
IV	14L/10D No Diamox	5	0.860±0.06	2.1±0.1	12.2±0.8	13.4±0.82	1.65±0.13	23.10±2.74

Table III. Part 1. Ocular parameters in chickens reared under two lighting conditions and given oral Diamox for six weeks. All values in this table and in subsequent tables are given as the mean ± standard error of the mean.

	Group	n	Volume of Aqueous Space (µ/liters)	Corneal Diameter (mm)	Corneal Height (mm)	Corneal Radius of Curvature (mm)	Equatorial Diameter of Eye (mm)	Meridonial Diameter of Eye (mm)
I	24L/0D Oral Diamox	5	33.7±2.6	7.2±0.1	1.5±0.1	3.8±0.2	17.5±0.2	13.4±0.2
II	24L/0D No Diamox	5	23.7±2.4	7.3±0.1	1.1±0.1	4.3±0.1	17.7±0.3	13.6±0.3
III	14L/10D Oral Diamox	5	62.5±1.8	8.0±0.1	2.2±0.0	3.5±0.0	16.8±0.1	12.6±0.3
IV	14L/10D No Diamox	5	63.5±2.8	8.0±0.1	2.3±0.1	3.5±0.0	17.1±0.3	12.5±0.1

Table IV. Part I. Ocular parameters in chickens reared under two lighting conditions and given oral Diamox for six weeks.

	Group	n	Body Weight (kg)	Eye Weight (gms)	IOP Experimental Eye (mmHg)	IOP Control Eye (mmHg)	Outflow Facility (µL/min)	Aqueous Inflow (µL/min)
I	24L/0D Oral Diamox	5	1.84±0.14	3.8±0.2	15.8±1.6	14.4±1.8	0.76±0.22	2.57±0.26
II	24L/0D No Diamox	5	3.10±0.12	4.8±0.3	19.1±0.5	18.4±0.5	0.30±0.08	8.21±1.23
III	14L/10D Oral Diamox	5	1.87±0.11	2.8±0.1	9.0±0.7	9.6±0.6	2.01±0.24	10.92±2.26
IV	14L/10D No Diamox	5	2.7 ±0.27	3.1±0.2	10.9±0.9	11.4±0.7	2.28±0.11	20.35±4.46

Table V. Part I. Ocular parameters in chickens reared under two lighting conditions and given oral Diamox for fourteen weeks.

	Group	n	Volume of Aqueous Space (µ/litres)	Corneal Diameter (mm)	Corneal Height (mm)	Corneal Radius of Curvature (mm)	Equatorial Diameter of Eye (mm)	Meridonial Diameter of Eye (mm)
I	24L/OD Oral Diamox	5	41.26± 2.18	8.4±0.2	1.2±0.2	6.8±0.1	21.1±0.2	15.7±0.5
II	24L/OD No Diamox	5	32.60± 2.29	9.8±0.2	1.1±0.0	6.4±0.2	22.9±0.4	16.7±0.2
III	14L/10D Oral Diamox	5	93.84± 8.07	9.2±0.2	2.5±0.2	5.9±0.0	19.7±0.4	15.6±0.3
IV	14L/10D No Diamox	5	108.56±10.46	9.8±0.2	2.6±0.1	5.0±0.0	19.8±0.3	15.5±0.3

Table VI. Part I. Ocular parameters in chickens reared under two lighting conditions and given oral Diamox for fourteen weeks.

Figure 2

A comparison of the IOP of 24L/0D and 14L/10D birds at 6 and 14 weeks of age. While the mean IOP at 6 weeks were almost identical, by 14 weeks of age the mean IOP in the 24L/0D birds was significantly higher than that of the 14L/10D group.

For all figures, the vertical bar represents standard error of the mean, the large 0, ●, ▲, or ■, indicates the mean, and small points represent individual values. In this series, n = 5 for each group.

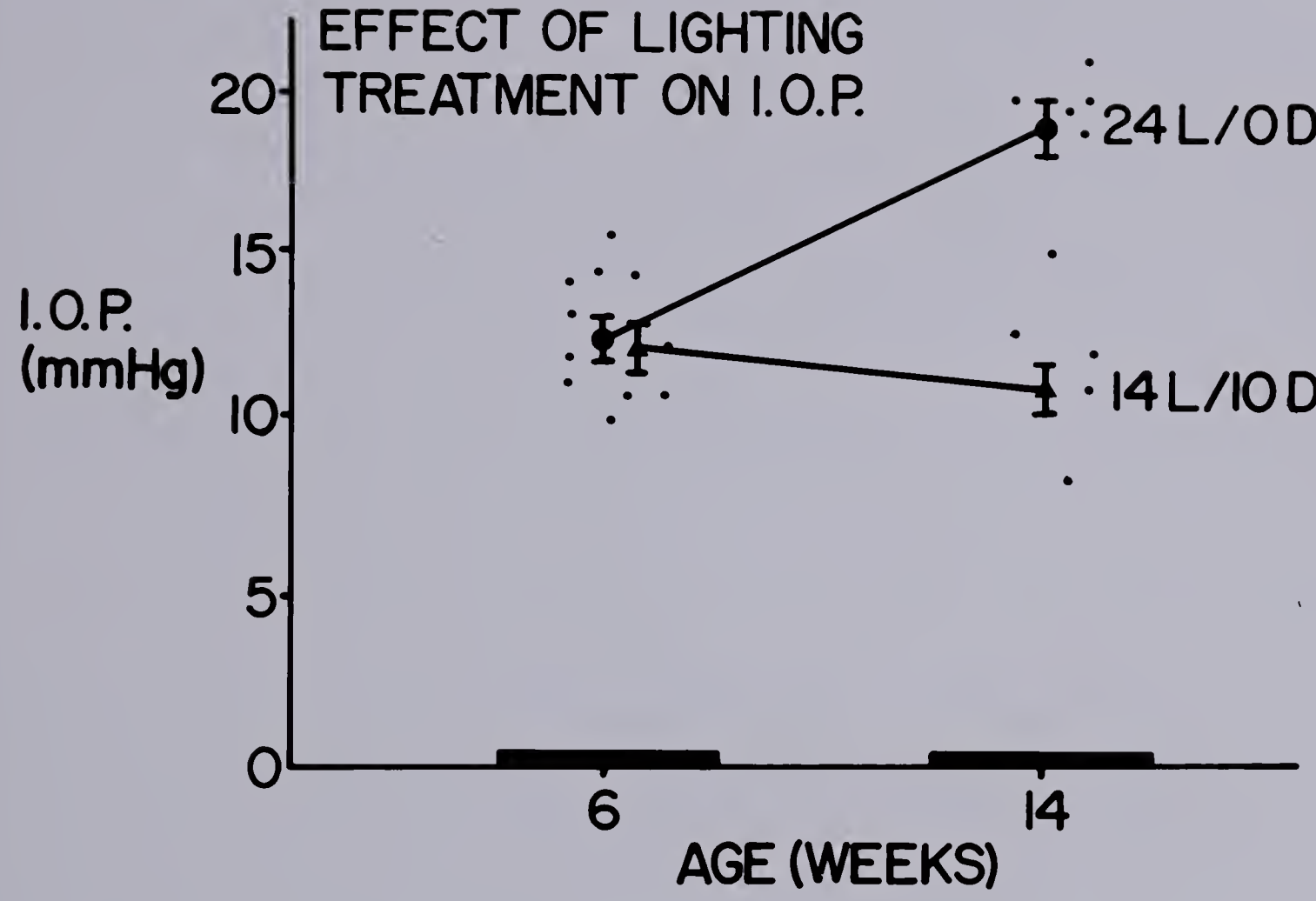

EFFECT OF LIGHTING
TREATMENT ON I.O.P.
I.O.P.
(mmHg)
20
15
10
5
0
24 L/0 D
14 L/10 D
6
14
AGE (WEEKS)

Figure 3

A comparison of IOP between 24L/OD birds fed Diamox and control 24L/OD birds fed a normal diet. While the mean IOPs at 6 weeks were almost identical, by 14 weeks the mean IOP in the Diamox-fed birds was lower than that in the control group. Although this difference is not statistically significant, the trend at 14 weeks may be correlated with lower mean eye weight (Fig. 6) and decreased aqueous flow (Fig. 7) in the Diamox-treated 24L/OD birds.

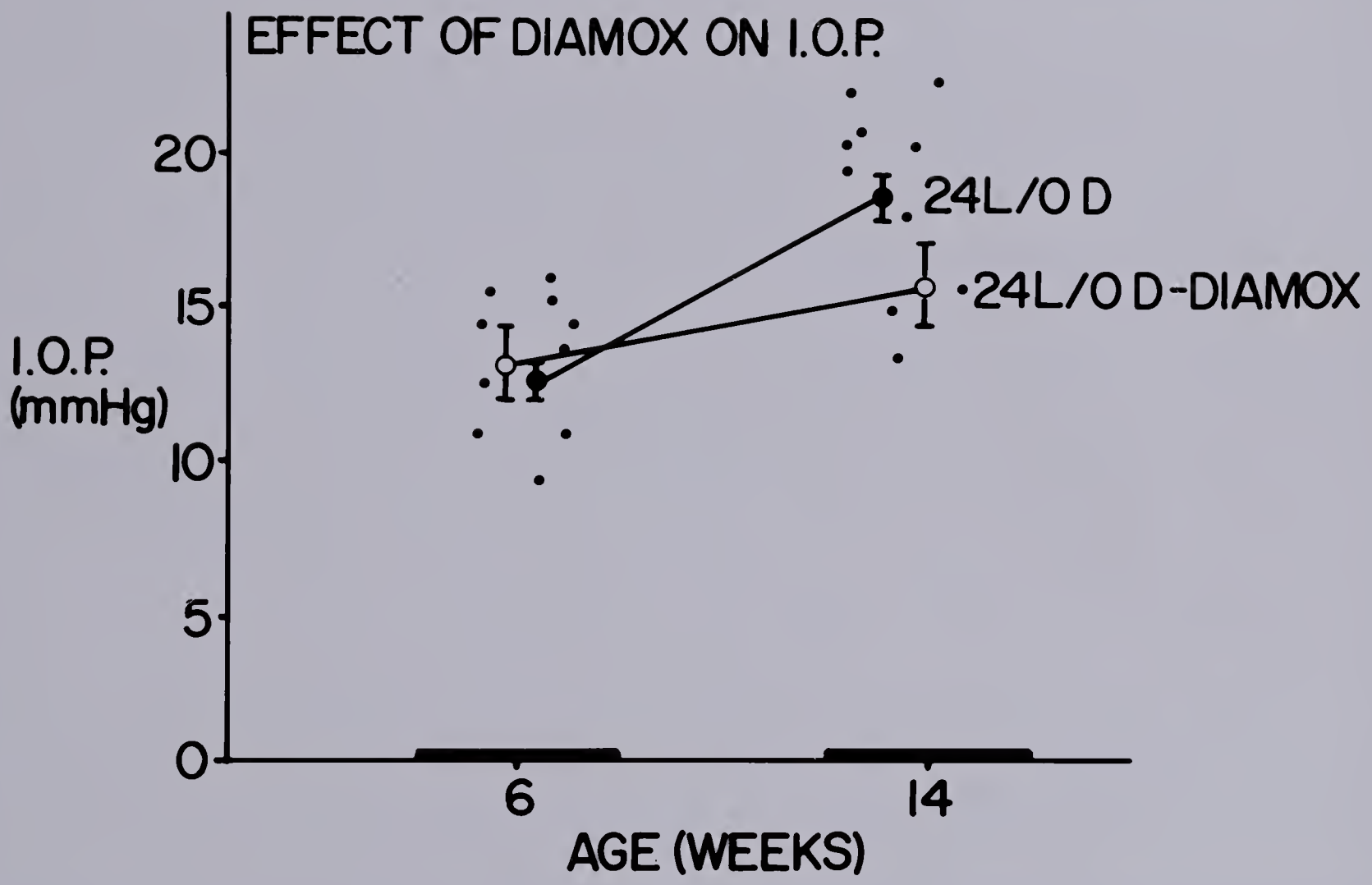

EFFECT OF DIAMOX ON I.O.P.
20
15
10
5
0
I.O.P. (mmHg)
24L/0 D
24L/0 D-DIAMOX
6
14
AGE (WEEKS)

Figure 4

A comparison of outflow facility (C) between the eyes of 24L/0D and 14L/10D birds at 6 and 14 weeks of age. While the mean C values at 6 weeks were almost identical, by 14 weeks the mean C of the 24L/0D birds was significantly lower than that of the 14L/10D birds. This low C is correlated with elevated IOP (Fig. 2) and impaired inflow (Fig. 7).

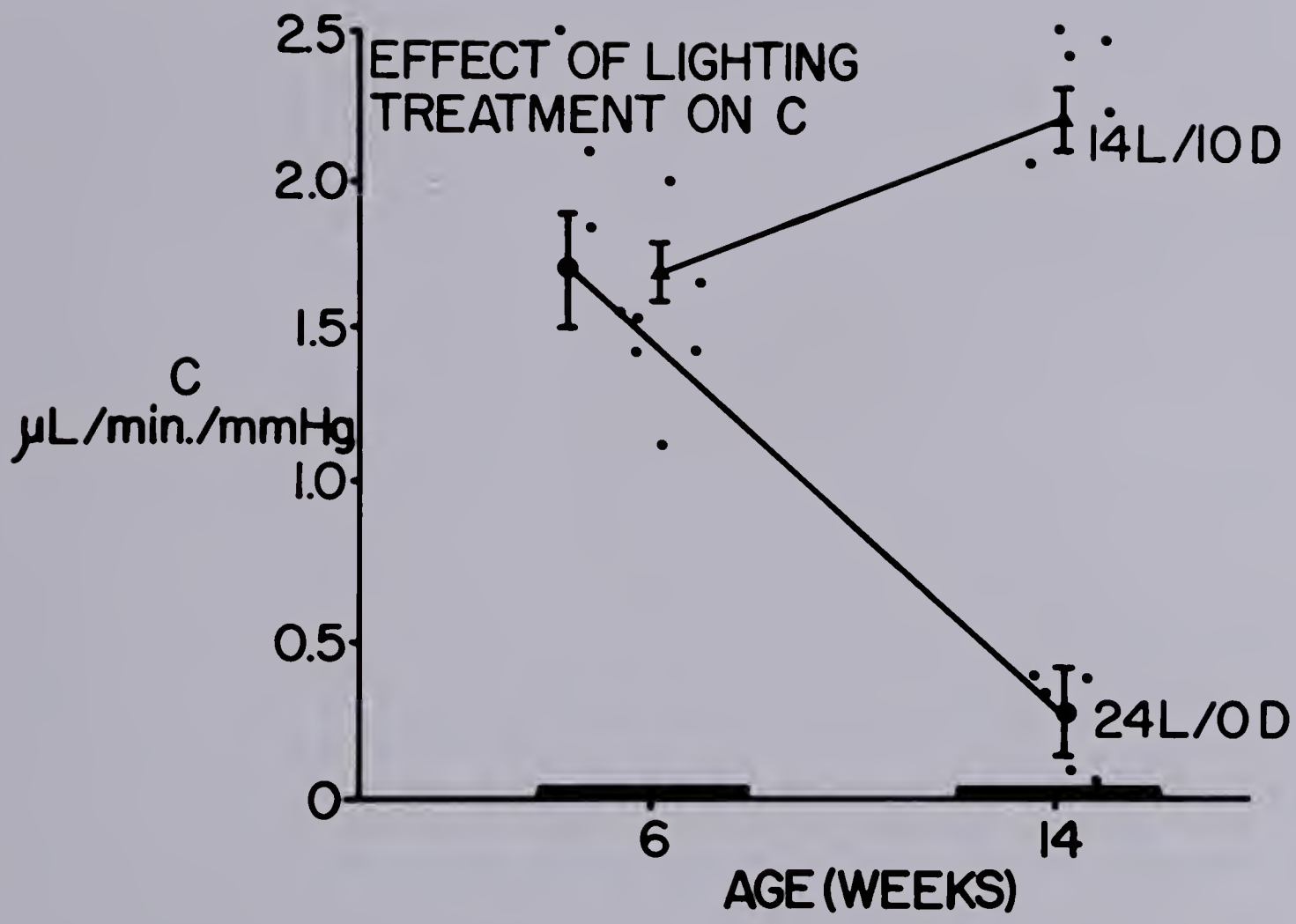

EFFECT OF LIGHTING TREATMENT ON C
2.5
2.0
1.5
1.0
0.5
0
C
µL/min./mmHg
14L/10D
24L/0D
6
14
AGE (WEEKS)

Figure 5

A comparison of the outflow facility (C) between 24L/0D chicks fed Diamox and 24L/0D controls fed a normal diet. The mean C of the Diamox-fed birds was lower than that of the control group at both 6 and 14 weeks of age. Although this difference is not statistically significant, the trend may be correlated with a lower mean eye weight (Fig. 6) and a decreased aqueous inflow (Fig. 7) in the Diamox-treated 24L/0D birds.

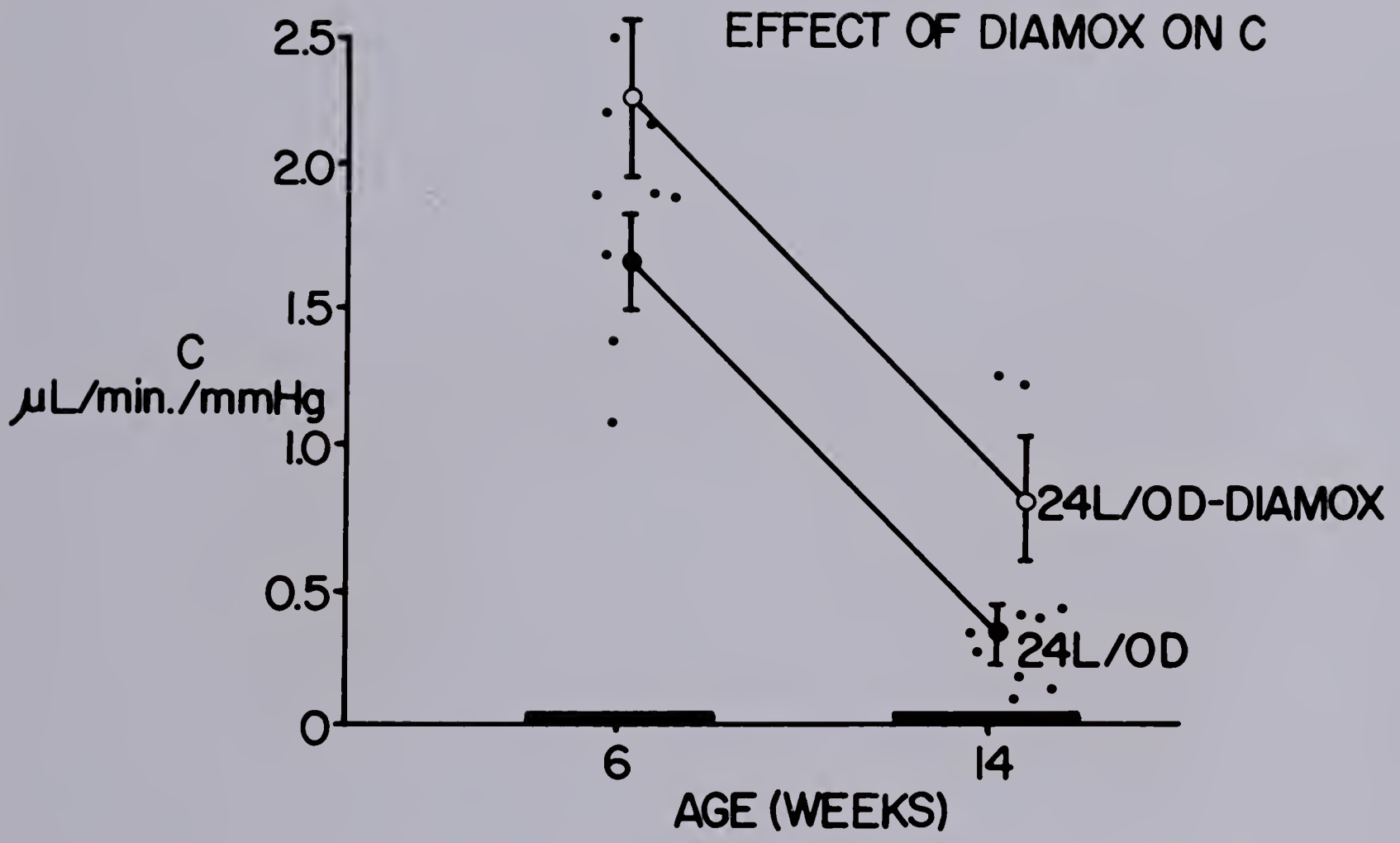

EFFECT OF DIAMOX ON C
2.5
2.0
1.5
C
µL/min./mmHg
1.0
0.5
0
24L/OD-DIAMOX
24L/OD
6
14
AGE (WEEKS)

Figure 6

A comparison of eye weight between 24L/0D birds fed Diamox and control 24L/0D birds fed a normal diet. While there was no difference between the groups at 6 weeks, by 14 weeks of age the mean eye weight of the Diamox-fed birds was significantly lower than that of the control 24L/0D birds, and was only slightly greater than that of 14L/10D birds.

This significantly lower eye weight is correlated with a significantly lower aqueous inflow (Fig. 7) and a tendency to lower IOP (Fig. 3) and higher C (Fig. 5) in the Diamox-treated 24L/0D birds.

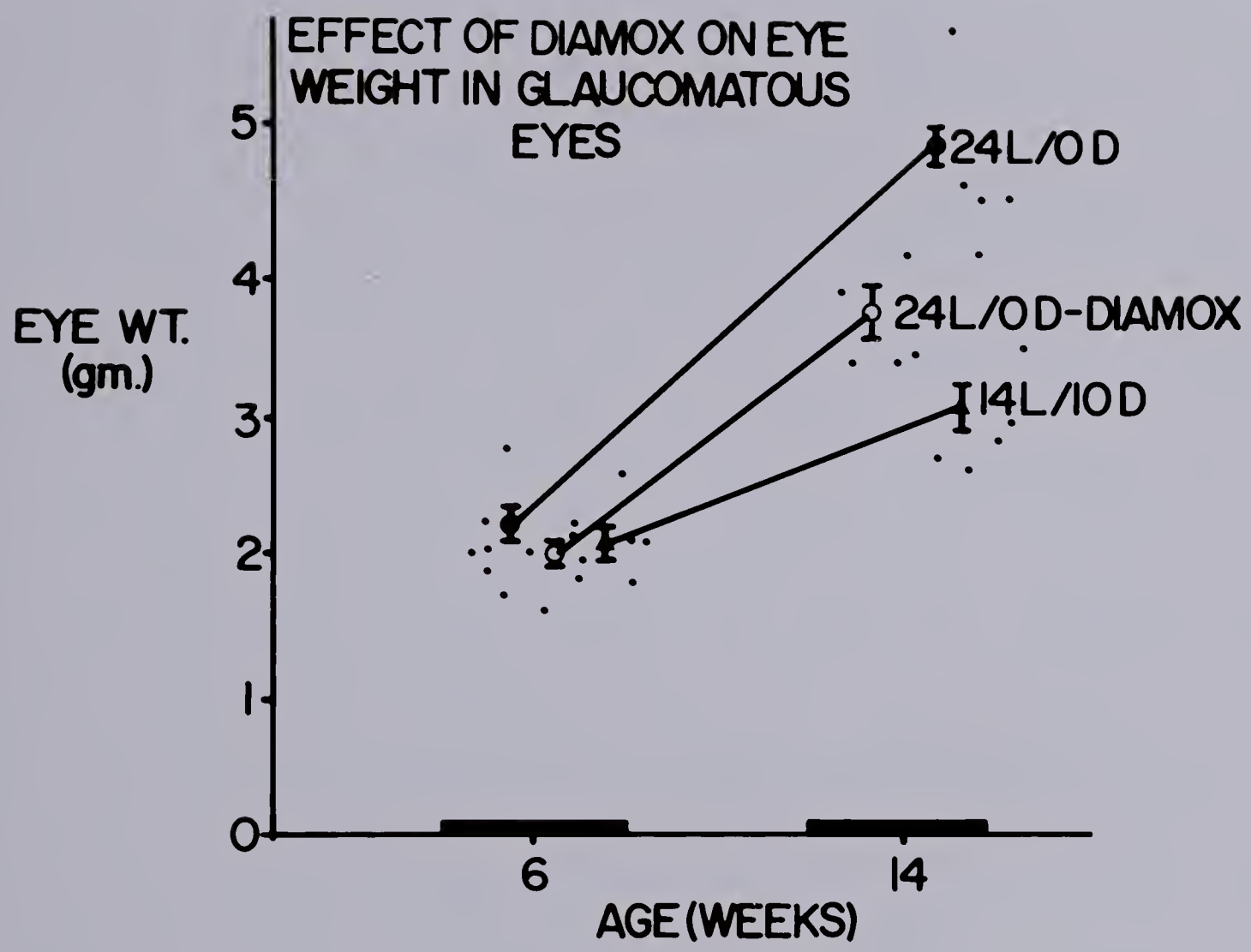
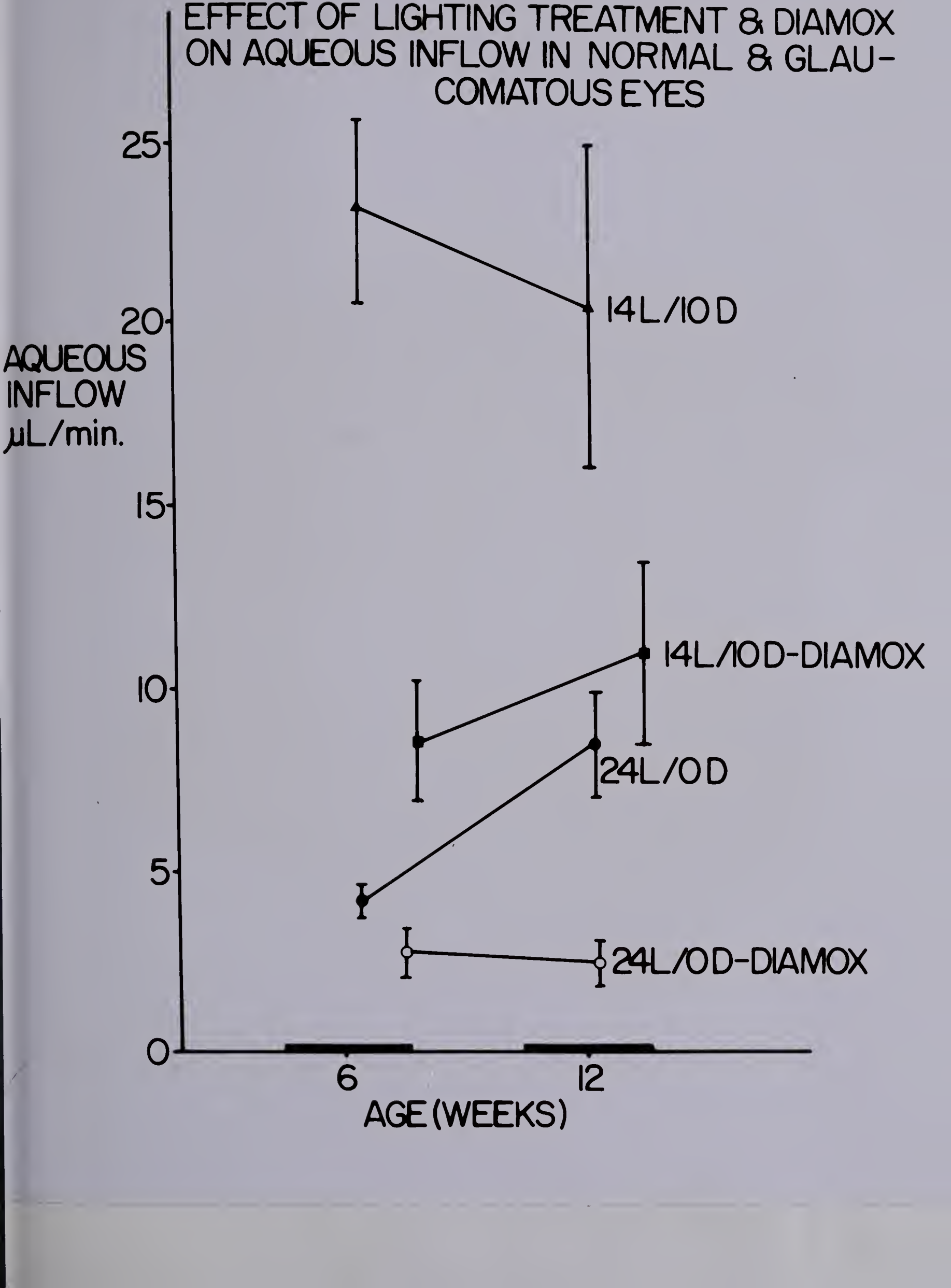

EFFECT OF DIAMOX ON EYE WEIGHT IN GLAUCOMATOUS EYES
EYE WT. (gm.)
5
4
3
2
1
0
24L/OD
24L/OD-DIAMOX
14L/IOD
6
14
AGE (WEEKS)

Figure 7

A comparison of aqueous inflow between lighting treatments and within lighting treatments in Diamox-fed chicks.

The mean aqueous inflow of the 24L/0D birds is significantly lower than that of the 14L/10D birds at both 6 and 14 weeks. This low inflow in 24L/0D birds is correlated with low C (Fig. 4), high IOP (Fig. 2), and greater eye weight (Fig. 6).

The expected effect of Diamox in decreasing aqueous inflow is shown by the significantly lower aqueous inflow values in the 24L/0D and 14L/10D birds fed Diamox as compared to their respective controls fed a normal diet.


EFFECT OF LIGHTING TREATMENT & DIAMOX ON AQUEOUS INFLOW IN NORMAL & GLAUCOMATOUS EYES
AQUEOUS INFLOW µL/min.
25
20
15
10
5
0
6
12
AGE (WEEKS)
14L/10D
14L/10D-DIAMOX
24L/0D
24L/0D-DIAMOX

Part II. The Role of Angle Closure in Light-Induced Avian Glaucoma.

It has previously been noted that the glaucoma-like condition which develops in chicks exposed to continuous light is associated with a shallow anterior chamber and a narrow iridocorneal angle.[68] If the angle were completely blocked, aqueous humor would have no means of exit from the eye and the IOP would increase.

The question thus arises as to whether the narrow iridocorneal angle is the primary mechanism responsible for the development of the glaucoma, or whether it is secondary to other changes occurring in the eye.

Smith *et al.*[93] suggest that the finding of a shallow anterior chamber, plus the gonioscopic appearance of a narrowed angle early in the disease, indicates that an angle closure mechanism was induced by the continuous light.

In humans with primary angle closure glaucoma, impaired drainage caused by pupillary block and iris bombé can be relieved by early iridectomy.

In this part of the study, iridectomies were performed on 3-day-old chicks subsequently reared under either diurnal or continuous lighting conditions.

If angle-closure were the primary mechanism responsible for the development of the light-induced avian glaucoma, it might be expected that iridectomy would prevent the glaucoma or alter its course, by making pupillary block impossible.

Materials and Methods

The experimental subjects were 20 male White Rock chicks randomly assigned to four equal groups as shown in Table VII. Food and water were supplied *ad lib*.

Under Combutal anesthesia[27], iridectomies were performed on the right eyes of Group I and Group III birds at 3 days of age. A Zeiss operating microscope was used to magnify the operating field.

The anterior chamber was entered at the limbus by an incision made with a keratome at the 9 o'clock position. The iris was prolapsed, grasped with forceps, and the iridectomy was performed with iridocapsulotomy scissors. The iris sphincter was thus cut, but no attempt was made to achieve basal iridectomy. Air was then injected to reform the anterior chamber, and no sutures were used.

The chicks recovered well from the operation: by the following day the anterior chamber had filled with aqueous and there was no sign at any time of severe inflammation or cloudiness of the cornea.

At 14 weeks of age the birds were anesthetized with intramuscular Nembutal (50 mgm/kg) and IOP, C, and aqueous flow were determined as previously described (Fig. 1).[65]

After removal of the cannulas, and when the anterior chambers had reformed, the animal was destroyed and the right eye enucleated and photographed for measurement of several dimensional parameters.

Results (Tables VIII and IX)

(1) In confirmation of previous findings in this and other studies, glaucoma was induced in chicks by continuous light.

Group II birds (24L/0D) as compared with Group IV (14L/10D) had significantly greater eye weight, flatter cornea, shallower anterior chamber, smaller volume of the aqueous space, larger equatorial and meridonial eye diameter, higher IOP, lower outflow facility, and reduced aqueous flow ($.01<p<.02$).

(2) Iridectomy did not prevent the development of glaucoma.

Group I birds (24L/0D-iridectomy) as compared to Group III birds (14L/10D-iridectomy) had significantly greater eye weight, smaller volume of the aqueous space, shallower anterior chamber, smaller corneal diameter, higher IOP, lower outflow facility, and reduced aqueous flow ($.01<p<.02$).

(3) Iridectomy did not alter the course of the glaucoma.

There were no significant differences at the 5% level between the eyes of Group I (24L/0D-iridectomy) and Group II (24L/0D) birds in any of the various ocular parameters measured except for a lower aqueous flow and smaller corneal diameter in Group I ($.01<p<.02$).

(4) The surgical procedure was not damaging. Comparisons between the operated right eye and its contralateral unoperated eye revealed no significant differences

in any of the ocular parameters within either lighting treatment except for the lower C in Group III (14L/10D-iridectomy) as compared to Group IV (14L/10D).

In addition, there were no findings at any time which would suggest an adverse effect from the iridectomy: the pupils responded rapidly to light, the corneas were clear, and the birds did not seem to be photophobic.

Conclusions

(1) A glaucoma-like condition was produced in chickens by exposing tyem to continuous light from the time of hatching.

(2) Iridectomy prior to continuous light treatment did not prevent the development of light-induced glaucoma and did not alter its course.

(3) Angle closure thus does not appear to be the primary cause of avian glaucoma, but it may be secondary to other factors.

The results of this part of the study are considered more fully in the General Discussion.

	Lighting Treatment*	Right Eye	Left Eye
Group I	24L/0D	Iridectomy Cannulation Infusion	Cannulation
Group II	24L/0D	Cannulation Infusion	Cannulation
Group III	14L/10D	Iridectomy Cannulation Infusion	Cannulation
Group IV	14L/10D	Cannulation Infusion	Cannulation

* 24L/0D = continuous light
14L/10D 14 hours light, 10 hours darkness

Table VII. A summary of experimental subjects for Part II.

	Group	n	Body Weight (kg)	Eye Weight (gms)	IOP Right Eye (mmHg)	IOP Left Eye (mmHg)	Outflow Facility (µL/min)	Aqueous Flow (µL/min)
I	24L/0D Operated	5	2.96±0.13	5.3±0.3	20.8±2.4	21.1±2.1	0.42±0.09	3.34±0.56
II	24L/0D Unoperated	5	3.10±0.12	4.8±0.3	19.1±0.5	18.4±0.5	0.30±0.08	8.21±1.23
III	14L/10D Operated	5	2.24±0.15	3.4±0.1	11.5±0.5	10.6±0.5	1.51±0.13 *	16.41±3.09
IV	14L/10D Unoperated	5	2.70±0.27	3.0±0.2	10.9±0.9	11.4±0.7	2.28±0.11 *	20.35±4.46

Table VIII. Part II. Ocular parameters in chickens reared under two lighting conditions and after iridectomy.

	Group	n	Volume of Aqueous Space (µ/litres)	Corneal Diameter (mm)	Corneal Height (mm)	Corneal Radius of Curvature (mm)	Equatorial Diameter of Eye (mm)	Meridonial Diameter of Eye (mm)
I	24L/0D Operated	5	36.37± 2.01	8.7±0.1	1.2±0.0	7.2±0.3	23.3±0.4	17.2±0.3
II	24L/0D Unoperated	5	41.26± 2.18	9.8±0.1	1.2±0.1	6.4±0.2	22.7±0.4	17.4±0.5
III	14L/10D Operated	5	100.92±11.08	9.6±0.2	2.5±0.2	5.0±0.0	21.8±0.6	16.0±0.4
IV	14L/10D Unoperated	5	108.56±10.46	9.8±0.2	2.6±0.2	5.0±0.0	19.8±0.3	15.5±0.3

Table IX. Part II. Ocular parameters in chickens reared under two lighting conditions and after iridectomy.

Part III-A. The Effect of Acute Intravenous Diamox on Intraocular Pressure, Aqueous Inflow and Aqueous Outflow Facility.

It is well established in the literature that Diamox, when administered to both humans and animals, decreases intraocular pressure (IOP) by decreasing the secretion of aqueous humor into the eye.[18,71] There has also been the occasional suggestion that Diamox may have some effect on aqueous outlfow facility (C).[45]

Increased outflow resistance has been shown after an induced inhibition of aqueous secretion in both human,[17,82] chicken,[88] and rabbit eyes.[87]

Sears[87] found that acute intravenous Diamox in the rabbit eye caused no change in IOP and was associated with significant decreases in aqueous flow and outflow facility. He also found that acute intravenous Diamox caused only a slight transient decrease in IOP in the unanesthetized chicken.

The purpose of this part of the study was to determine the effect of acute intravenous Diamox on IOP, aqueous inflow (F) and aqueous outflow facility (C) in both normal and glaucomatous avian eyes.

Materials and Methods

The experimental subjects were 10 White Rock male chicks randomly assigned to two equal groups as shown in Table X.

At 14 weeks of age each bird was anesthetized with intramuscular nembutal (50 mgm/kg). Both eyes were cannulated, and then allowed to stabilize for 1 hour. IOP was monitored and C was measured as previously described.[65] The animal was again left to stabilize for 1 hour and the following procedures were carried out.

(1) One cc of 10% sodium fluorescein in normal saline was injected intravenously (i.v.), and the build-up of fluorescein in the anterior chamber was recorded. After a good inflow curve was developing, and while IOP was being monitored, Diamox (125 mgm in 1.25 cc water) was slowly given i.v. over a one minute period. Any changes in flow and IOP were recorded during the next 10 minutes.

(2) Ten minutes after the injection of Diamox, C was again measured.

(3) The cannulas were then removed and the anterior chambers were allowed to reform. The right eye was then enucleated, trimmed of excess fat and muscle, weighed and photographed for later measurement of several parameters.

Results and Conclusions (Tables XI and XII)

(1) Glaucoma was induced in chicks by continuous light. The 24L/0D chicks as compared to the 14L/10D group had significantly greater eye weight ($.01<p<.02$) and IOP ($.02<p<.05$) and significantly lower C, shallower anterior chamber, flatter cornea, smaller volume of the aqueous space, greater equatorial eye diameter, and lower aqueous flow ($.001<p<.01$).

(2) In confirmation of previous findings,[87] and as was predicted, acute i.v. Diamox caused a marked decrease in aqueous inflow in both Group I and Group II animals ($.01<p<.02$).

(3) There was a marked difference between the two groups in the effect of acute i.v. Diamox on IOP.

In glaucomatous birds, the drug caused a significant decrease in IOP ($.01<p<.02$) which was immediate, marked, and sustained. Normal chicks (14L/10D), however, displayed an immediate but only transient decrease in IOP, which rapidly (within 2 minutes) returned to pre-treatment levels in all cases.

(4) Acute i.v. Diamox caused a marked and opposite effect on C in the two groups.

Ten minutes after the drug was given, 24L/0D birds had a significantly greater mean C value ($.01<p<.02$) as compared to their own pre-treatment level, while the 14L/10D birds showed a significant decrease ($.02<p<.05$) in C as compared to pre-treatment levels.

	Lighting Treatment*	Right Eye	Left Eye
Group I	24L/0D	Cannulation Infusion	Cannulation
Group II	14L/10D	Cannulation Infusion	Cannulation

*24L/0D = continuous light
14L/10D - 14 hours light, 10 hours darkness

Table X. A summary of experimental subjects for Part III-A.

	Group	n	Body Weight (kg)	Eye Weight (gms)	Volume of Aqueous Space (µ/litres)	Corneal Diameter (mm)	Corneal Height (mm)	Radius of Curvature (mm)	Equatorial Diameter of Eye (mm)	Meridonial Diameter of Eye (mm)
I	24L/0D	5	2.1 ±0.17	4.0±0.4	37.86±2.74	9.0±0.1	1.2±0.1	7.6±0.4	21.9±0.5	16.3±0.7
II	14L/10D	5	1.94±0.16	2.6±0.7	87.11±8.55	8.9±0.2	2.5±0.2	4.6±0.3	18.9±0.0	15.3±0.2

Table XI. Part III-A. Ocular parameters of chickens reared under two lighting conditions and after acute intravenous Diamox.

	Group	n	IOP Before iv Diamox* (mmHg)	IOP After iv Diamox** (mmHg)	Outflow Facility Before iv Diamox* (µL/min)	Outflow Facility After iv Diamox** (µL/min)	Aqueous Flow Before iv Diamox* (µL/min)	Aqueous Flow After iv Diamox** (µL/min)
I	24L/0D	5	29.3±4.7	16.8±1.0	0.13±0.03	0.85±0.23	5.5±0.5	2.0±0.4
II	14L/10D	5	14.3±1.4	13.5±1.6	2.32±0.30	1.36±0.27	6.6±2.2	3.3±1.2

* Diamox = Acetazolamide, 125 mgm in 125 cc sterile water
** 10 minutes after iv injection of Diamox

Table XII. Part III-A. Ocular parameters of chickens reared under two lighting conditions and after acute intravenous Diamox.

Part III-B. The Effect of Acute Intravenous Diamox on Systemic Arterial Blood Pressure.

It seemed possible that acute i.v. Diamox may have caused a decrease in aqueous secretion by reduction of blood flow through the ciliary body, secondary to decreased systemic arterial blood pressure (B.P.).

Sears[88] found that acute i.v. Diamox produced an immediate and brief decrease in systemic arterial B.P. in 4 out of 6 unanesthetized hens.

The purpose of the present study was to determine if the decrease in aqueous inflow caused by acute i.v. Diamox was associated with any change in systemic arterial blood pressure.

Materials and Methods

The experimental subjects were 2 male White Rock chickens. Animal I was reared in continuous light (24L/0D) and animal II was reared in diurnal light (14L/10D).

At 18 weeks of age the birds were anesthetized with i.m. Nembutal (50 mgm/kg), the eyes were cannulated and IOP was monitored as previously described.[65] A plastic catheter filled with heparinized normal saline was inserted into a wing artery and was connected to a Statham p. 23 pressure transducer which was in turn connected to a Beckman Dynagraph recorder. This system was used to monitor systemic arterial blood pressure.

Diamox (125 mgm in 1.25 cc water) was then slowly injected into a wing vein over 1 minute, and any changes in B.P. and IOP were recorded over the next 20 minutes.

Results and Conclusions (Table XIII)

(1) In confirmation of previous findings, (Part III-A) acute i.v. Diamox caused an immediate, marked, and sustained decrease in IOP in the glaucomatous bird (animal I) and an immediate but transient decrease in IOP in the normal bird (animal II).

(2) Acute i.v. Diamox caused no changes in systemic arterial blood pressure in either bird during the 20 minute observation period following injection.

	Animal	n	IOP Before iv Diamox* (mmHg)	IOP After iv Diamox** (mmHg)	Arterial B.P. Before Diamox* (mmHg)	Arterial B.P. After Diamox** (mmHg)
I	24L/0D	1	23.0	13.0	87.0	89.0
II	14L/10D	1	12.5	12.0	90.0	89.0

* Diamox = Acetazolamide 125 mgm
** 10 minutes after iv injection of Diamox

Table XIII. Part III-B. Changes in intraocular pressure and arterial blood pressure in two chickens reared under two lighting conditions after acute intravenous Diamox.

Part III-C. The Effect of Acute Intravenous Diamox on The Outflow Mechanism of the Eye.

As noted in Part III-A of this study, acute i.v. Diamox produced increased outflow facility (C) in chicks with light-induced avian glaucoma (24L/0D), and a decrease in C in normal birds (14L/10D). The increased C in glaucomatous birds after i.v. Diamox was associated with a marked and sustained drop in IOP and a decrease in aqueous inflow, while the decrease in C in the normal birds after i.v. Diamox was associated with only a transient drop in IOP and a decrease in aqueous inflow.

In an effort to determine if these changes in C were secondary to the decrease in aqueous inflow, the following study was done.

Materials and Methods

Two chicks from each lighting treatment were assigned to one of 2 groups as shown in Table X.

At 18 weeks of age, each chick was anesthetized with i.m. Nembutal (50 mgm/kg), the eyes were cannulated and measurement of IOP and C were carried out as previously described.

Preliminary studies in this lab had shown that aqueous inflow in the chicken eye ceased when the IOP was artificially increased to a level of 40-45 mmHg.[66]

In this study, the assumption was made that if the IOP was artificially increased to a new steady level greater

than 45 mmHg by infusing saline into the eye at a constant high rate, aqueous inflow would be effectively shut off. Any changes in IOP which then occurred subsequent to i.v. injection of Diamox would be due to changes in the outflow mechanism of the eye caused by some factor or factors other than aqueous inflow.

After C was measured, the IOP was increased to a new steady level greater than 50 mmHg by infusion of normal saline into the eye at a constant high rate.

One cc of 10% fluorescein was then injected into a wing vein and aqueous inflow was monitored, as previously described,[65] in order to make sure that there was no aqueous inflow present at the artificially increased IOP. If any fluorescein was detected in the anterior chamber, it would mean that some aqueous inflow was still occurring.

Diamox (125 mgm in 1.25 cc water) was then given slowly i.v. over 1 minute and any changes in IOP were noted over the next 10 minutes. As aqueous inflow into the eye had ceased at the time when Diamox was given, any changes in IOP that occurred would be a result of some alteration in the outflow mechanism.

Results and Conclusions (Table XIV)

(1) There was no aqueous inflow detected in any of the experimental subjects while the IOP was artificially maintained above 45 mmHg.

(2) Acute i.v. Diamox caused an immediate and sustained decrease in IOP in the glaucomatous chicks (24L/0D), even though the eye was being perfused with normal saline at a constant rate and aqueous inflow was thus shut off.

This suggests that there was some improvement in the facility of outflow in these glaucomatous eyes after the administration of acute i.v. Diamox, and it also suggests that this change in facility is due to other factors besides a decrease in aqueous inflow.

(3) There was no alteration of IOP in Group II (14L/10D) birds after the injection of i.v. Diamox. This suggests that there was no change in C following Diamox injection in normal chickens when aqueous inflow is shut off, and that the decrease in C following acute i.v. Diamox injection that was found in Part III-B in 14L/10D birds may be secondary to a decrease in aqueous inflow.

This is only a small series, however, and a large experiment is needed in order to obtain statistically significant data.

	Animal	IOP During Infusion Before iv Diamox* (mmHg)	IOP During Infusion After iv Diamox** (mmHg)
I	A 24L/0D	60.0	50.0
I	B 24L/0D	58.0	47.0
II	A 14L/10D	57.0	55.0
II	B 14L/10D	49.0	51.0

* Diamox = Acetazolamide 125 mgm
** 10 minutes after iv injection of Diamox

Table XIV. Part III-C. Changes in intraocular pressure during constant rate saline infusion following acute intravenous Diamox in chickens reared under two lighting conditions.

VI. DISCUSSION

The purpose of this series of experiments was to search for the primary mechanisms responsible for the development of light-induced avian glaucoma.

The main facts that are known about this condition at the present time are:

(1) Chickens reared under continuous light develop a glaucoma-like condition.[55,69]

(2) The glaucoma is characterized by increased intraocular pressure, decreased aqueous outflow facility, increased eye weight, eye enlargement, reduced corneal curvature, shallow anterior chamber, and narrow iridocorneal angle.[68,93]

The development of the glaucoma may be due to a local ocular effect of light or to a general systemic effect of light which in turn causes some alteration in aqueous fluid dynamics.

Smith *et al.*[93] felt that the finding of a shallow anterior chamber plus the gonioscopic appearance of a narrowed iridocorneal angle early in the disease process indicated that an angle closure mechanism is induced by the continuous light, and that this subsequently causes the increased IOP and eye enlargement.

The fact that the eye changes are induced by modification of the environment suggests that a systemic response could be involved. A general, rather than a localized response to light was also invoked by Lauber[67] to explain

the development of light-induced buphthalmos in spite of covering the eye with an occluder from hatching to six weeks of age.

Several other photosensitive parameters in birds and mammals are well known. In particular, the reproductive system,[39] pituitary and hypothalamus,[79] adrenal cortex,[85] pineal body,[6,29] as well as behavior,[75] are all known to be profoundly affected by the photoperiod.

It is also well established that there is a diurnal variation in IOP in both normal and glaucomatous human eyes.[32] These variations are thought to be due primarily to alterations in the secretion of aqueous humor.[38] The influence of light on aqueous fluid dynamics may be mediated by some photosensitive endocrine or neural mechanism.

It seems possible that avian glaucoma, which is brought on by abolishing the day-night cycle, might be associated with an alteration in aqueous secretion rate caused by a neuro-endocrine mechanism.

Unpublished studies by Lauber, Boyd, and Boyd,[66] on chicks exposed to continuous or diurnal light treatment from hatching and examined during the first few weeks of life, indicate that aqueous inflow and outflow facility are at first higher in the 24L/0D eyes than in 14L/10D eyes. These studies also show that by 6 to 8 weeks of age the C of the 24L/0D birds has dropped below the control level. This decrease in C in the 24L/0D birds is followed in 4 to 6 weeks by a dramatic increase in IOP.

If hypersecretion of aqueous humor were responsible for the development of light-induced avian glaucoma, it might be expected that oral Diamox given during the period of continuous light treatment would prevent the glaucoma from developing or would alter its course.

The glaucoma which developed in the Diamox-treated 24L/0D chicks was less severe than that which developed in the untreated control 24L/0D birds. This is shown by the significantly lower eye weight, smaller eye dimensions and corneal diameter, and by the significantly larger corneal height and volume of the aqueous space in the Diamox-fed 24L/0D chickens.

The IOP tended to be lower and the C tended to be greater in the Diamox-treated 24L/0D birds. Even though these differences were not statistically significant, they were associated with a significantly lower eye weight and aqueous inflow in the Diamox-treated birds.

By 14 weeks of age, severe glaucoma had developed in all 24L/0D birds and they had significantly greater eye weight, higher IOP, larger eyes, lower C, lower aqueous inflow, smaller volume of the aqueous space and flatter corneas, as compared to 14L/10D birds.

One can surmise that the lower aqueous inflow found in the 24L/0D birds at 6 weeks may have been the result of alterations in some homeostatic mechanism controlling IOP. The outflow mechanism may be so damaged after 6 weeks of continuous light exposure that it cannot respond to increases

in inflow by increases in outflow and IOP is gradually elevated. This increase in IOP may in turn lead to a decrease in inflow and the normal IOP which is seen in 24L/0D chickens at 6 weeks of age is the end result. This assumed homeostatic mechanism seems to have its limitations, however, and by 14 weeks of age it is obvious that it has failed and there has been an uncompensated increase in IOP, leading to further eye changes.

Part II of this study was concerned with the role of angle closure in light-induced avian glaucoma. As previously noted, avian glaucoma is associated with the early development of a shallow anterior chamber, narrow iridocorneal angle, and decreased volume of the aqueous space.

Smith *et al.*[93] concluded from their experiments that an angle closure mechanism is primarily responsible for the development of light-induced avian glaucoma. They felt that continuous light induces an anatomical change in the anterior chamber which subsequently cause increased IOP and enlargement of the eye.

In this part of the study, iridectomies were performed on 3-day-old chicks subsequently reared under either continuous or diurnal lighting conditions.

If angle closure were the primary mechanism responsible for the development of light-induced avian glaucoma, it was felt that iridectomy would prevent the glaucoma or alter its course by making pupillary block impossible.

The results showed that iridectomy did not prevent the

glaucoma or alter its course. The glaucoma which developed under 24L/0D was as severe in those birds subjected to iridectomy as in those birds on whom no surgery was performed. These results suggest that angle closure is not the primary cause of this condition, although impaired drainage may secondarily contribute to further deterioration of aqueous flow. Angle closure may be secondary to other factors, including possibly an increase in aqueous humor secretion during the early weeks of continuous light exposure.

Other factors besides hypersecretion of aqueous humor may be responsible for light-induced avian glaucoma. There may be some other change in the eye, such as an alteration in structure or function of the trabecular meshwork, of the aqueous veins, or the venous drainage of the eye. There may also be some change in the chemical or physical properties of the aqueous humor, such as increased solute or increased viscosity of the fluid, effecting a secondary change in aqueous fluid dynamics. Further experiments are needed to test these theories.

The purpose of Part III was to determine the effect of acute intravenous (i.v.) Diamox on aqueous fluid dynamics in normal and glaucomatous chicken eyes.

Many authors feel that there is a homeostatic mechanism present which maintains IOP within a certain normal range.[5,36,38,62,82] They feel that there is a definite relationship between aqueous inflow and outflow, and that alterations in

one, caused by alterations in the other, will tend to maintain IOP within certain limits.

There is evidence to suggest that the human eye with primary open angle glaucoma cannot respond as effectively as the normal eye to changes in aqueous humor flow. Such patients show marked diurnal variation in IOP, while in the normal eye, diurnal changes in IOP are very small. These diurnal changes in IOP are thought to be due mainly to changes in aqueous inflow, which is high during the night and decreases during the waking hours.[32]

Patients with primary open angle glaucoma usually show impaired outflow facility, and they develop pathological changes in the trabecular meshwork.[54] Because of this damage, the eye cannot respond to the increased inflow that occurs during the night and the IOP will increase.

Other evidence for the presence of a regulatory homeostatic mechanism for IOP is the fact that normal human and rabbit eyes show a decrease in C after the acute injection of i.v. Diamox.[87] This decreased C is associated with a marked decrease in aqueous inflow, and only transient changes in IOP.

Recent evidence in the literature[45] has shown that patients with primary open angle glaucoma, undergoing long term oral Diamox therapy, show improvement in C associated with decreased aqueous inflow and IOP. This suggests that there may be some alteration in the homeostatic mechanism controlling such a glaucomatous eye. Specifically, the eye

appears to react to a decreased inflow by an increase in C.

The results of the present study are in agreement with other evidence that suggests the presence of homeostatic mechanism for the control of IOP in the normal eye, and also show that there is some alteration in IOP control in light-induced avian glaucoma.

Normal chickens reacted to acute i.v. Diamox by a marked decrease in aqueous inflow, marked decrease in C, and only transitory changes in IOP.

Glaucomatous chickens, however, reacted to acute i.v. Diamox by a marked decrease in aqueous flow, marked increase in C, and an immediate and sustained decrease in IOP. The fact that the same drug in the same dosage given to normal birds had a different effect in glaucomatous chicks suggests that the 24L/0D condition involves some alteration in the homeostatic mechanism for the control of IOP.

Pilot studies were done in Part II-B, to determine whether acute i.v. Diamox caused any alterations in systemic arterial blood pressure which might lead to secondary changes in IOP and aqueous flow. The results of this study showed that the drug had no effect on blood pressure in either the glaucomatous or normal bird during the 10 minute observation period. However, a larger experiment should be done to obtain results which could be statistically analyzed.

Pilot studies were also done in Part III-C to see if the effects of acute i.v. Diamox on C were primarily due to some pharmacological effect of the drug on the outflow

mechanism, or if they were secondary to changes in aqueous inflow. Injection of Diamox while aqueous inflow was shut off caused a decrease in IOP in glaucomatous eyes, even though these eyes were being perfused at a constant rate with normal saline. This suggests that the i.v. Diamox caused some alteration in C which was not secondary to a decreased aqueous inflow.

Normal chicks studied in the same manner showed no alterations in IOP after i.v. injection of Diamox. This suggests that the changes in C which were previously seen in Part III-A were secondary to a decreased aqueous inflow.

These pilot studies utilized only small numbers of animals, and larger series are needed to obtain results to which statistical procedures can be applied.

VII. CONCLUSIONS

(1) Chickens reared under continuous light from hatching develop a glaucoma-like condition. Hypersecretion of aqueous humor during the early weeks of life may be a primary mechanism responsible for the development of light-induced avian glaucoma. Subsequently the condition is characterized by eye enlargement, increased intraocular pressure, decreased outflow facility, and decreased aqueous inflow, flattening of the cornea with a shallow anterior chamber, and decreased volume of the aqueous space.

(2) Angle closure does not appear to be the primary cause of light-induced avian glaucoma.

(3) There appears to be a homeostatic mechanism present in the normal avian eye which compensates for changes in aqueous inflow by altering aqueous outflow, in order to keep IOP within a certain normal range. Light-induced avian glaucoma appears to involve alterations in the function of this homeostatic mechanism.

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